Supplemental Materials

Sirtuin 5 is regulated by the SCF-Cyclin F ubiquitin ligase and is involved in cell cycle control

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Supplemental Table 1. DRYGIN reported genetic interactions of *hst3*

Supplemental Table 2.

A. Phospho-proteomics of 293T SIRT5 crWT and crKO cells.
B. Total protein of 293T SIRT5 crWT and crKO cells.

Supplemental Figure 1 *Cdh1* deletion does not suppress the *cdc4-1* mutant phenotype.

A. wt, *cdc4* temperature sensitive mutant, *cdh1* deletion, or *cdc4cdh1* yeast were grown at 23°C, 26°C and 28°C for 3 days, while all other temperature incubations were 2 days.

Supplemental Figure 2 Proximity ligation assay confirms interaction between SIRT5 and Cyclin F

A. Representative images of Proximity Ligation Assay using U2OS cells co-expressing SIRT5-HA and Myc-Cyclin F. Tubulin= Green, PLA signal= Red.

Supplemental Figure 3 SIRT5 crKO cells with a shorter G1 phase load MCMs faster than WT SIRT5 cells

A. SIRT5 crWT and crKO cells were treated in EdU 30 minutes before collection, extraction of chromatin bound proteins and fixation in 4% PFA. Cells were stained for EdU (Alexa flour 647), loaded MCM2 (Alexa fluor 488) and DAPI. Samples were analyzed via flow cytometry to determine MCM loading (top row) and DNA synthesis (EdU incorporation; bottom row).

B. Percentage of cells from (A) in each cell cycle phase.

C. Histogram of G1-MCM^{DNA}-positive cells from (A). Gray – SIRT5 crWT; blue – SIRT5 crKO. Higher density of cells to the right indicates faster MCM loading.

Supplemental Figure 4 SIRT5 protein levels increase in arrested/quiescent cells

A. NHF cells were grown in various conditions to induce quiescence. Control cells were grown in normal complete medium. Serum starved cells were serum starved for 5 days. Contact inhibition was achieved by allowing cells to grow to confluency and stay at maximum confluency for 4 days.

B. RPE1 cells were grown in media containing 0.1% FBS for 72h hours before being released into media containing 10% FBS. Samples were collected at the indicated time points after addition of 10% FBS.

Supplemental Figure 5 CDK2 targets phosphorylation is increased in SIRT5 crKO cells

A. Phospho-peptides mapping to CDK2 targets CDC6, ORC6 and RB1 are significantly (p-value < 0.05) increased in SIRT5 crKO cells compared to crWT. Two different peptides mapping to the same RB1 site (T373) were identified, with individual sites being represented by two different points.